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Rapid Report

Plasma cholesterol esterase level is a determinant for an atherogenic lipoprotein profile in normolipidemic human subjects

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Abstract

Plasma cholesterol level is controlled by various factors. In the present study, high plasma activity of cholesterol esterase was found to correlate with plasma total cholesterol and low density lipoprotein (LDL) cholesterol levels in normolipidemic human subjects. However, the cholesterol esterase is not elevated in plasma of patients with familial hypercholesterolemia. These observations suggest that cholesterol esterase level is not determined by plasma cholesterol level, but elevated cholesterol esterase may be causative in increasing plasma cholesterol and LDL. Additional experiments further demonstrated that cholesterol esterase can convert the larger and less-atherogenic LDL to the smaller and more atherogenic LDL subspecies in vitro. These results suggest that plasma cholesterol esterase contributes to the formation and accumulation of atherogenic lipoproteins, and thus is a major risk factor for premature atherosclerosis in normal human subjects.

Keywords: Low density lipoproteins; Atherosclerosis; LDL subspecies; Cholesterol esterase; Cardiovascular risk factor

Coronary heart disease is the leading cause of death in the Western world. This is a complex disorder involving many genetic and environmental factors [1–3]. A major risk factor associated with this disease is the elevated levels of total cholesterol and low density lipoprotein (LDL) cholesterol in plasma [4]. The most widely characterized genetic influence on LDL level is the abnormality associated with LDL receptor defects [5]. Mutations in the apolipoprotein B-100 gene have also been shown to affect LDL catabolism resulting in accumulation of LDL in plasma [6–9]. However, defective LDL receptor gene affects 1 in 500 individuals and the prevalence of defective apolipoprotein B-100 was estimated to be 0.2% [9–11]. Therefore, these rare genetic diseases cannot account for most of the variations in LDL level among the general population. Additional factors may also be involved in controlling LDL homeostasis.

Recent studies suggested that LDL particle size is an important risk factor for atherosclerosis. The results were supported by observations of association between individuals with predominantly small, dense LDL particles and their risk of premature atherosclerosis [12–14]. Based on LDL sizing by nondenaturing gradient gel electrophoresis, two distinct LDL subclass phenotypes have been described [13,15]. The LDL subclass phenotype B, characterized by a predominance of small LDL particles, is present in approx. 30% of the general population and is associated with increased risk of atherosclerosis [14,15].

Despite the current intense focus on understanding LDL metabolism and the identification of LDL size as a major risk factor for atherosclerosis, the enzyme(s) responsible for dictating LDL phenotype and atherosclerosis susceptibility has not been identified to date. The study by Zambon et al. showed an inverse relationship between hepatic lipase activity with LDL particle size [16]. However, hepatic lipase activity was found to be similar between normolipidemic subjects and coronary artery disease patients [16]. Although the importance of hepatic lipase as a risk factor for atherosclerosis remains uncertain, these results suggest that LDL phenotype may be dictated by lipolytic enzymes that can alter the lipid composition of lipoproteins.

Recently, the pancreatic cholesterol esterase, also called carboxyl ester lipase or bile salt stimulated lipase, has been shown to be synthesized and secreted into plasma by the liver [17–21]. Since this protein is a lipolytic enzyme with

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Table 1
Cholesterol esterase activity in human plasma of different normolipidemic human subjects

Subjects	Gender	Experiments	Cholesterol esterase activity [(pmol/h ng protein)$\times 10^5$]
1	F	A	4.20
		B	4.24
2	M	A	5.43
		B	7.62
3	F	A	3.00
		B	2.29
4	M	A	8.12
		B	7.94
		C	8.14
5	F	A	4.16
		B	5.46

Experiments A, B, and C indicated the samples were obtained at different days. Cholesterol esterase activity was measured based on the hydrolysis of cholesteryl [^{14}C]oleate. The data represented three separate determinations.

wide substrate specificity [reviewed in [22]], the cholesterol esterase in plasma may alter lipid content and change the surface-to-core lipid distribution and size of lipoproteins. The purpose of this investigation is to ascertain the possible correlation between plasma cholesterol esterase activity with cholesterol level and its effect on LDL particle size.

Initial experiments were performed to verify the presence of cholesterol esterase activity in human plasma. In these experiments, lipoprotein-free plasma were used to avoid possible lipoprotein interference of the in vitro assay. The results showed that consistent cholesterol esterase activity could be obtained from different sampling of the same subject if blood was drawn in the morning after an overnight fast (Table 1). These results indicated that potential variations in cholesterol esterase activity among various plasma samples [17] were not due to variability in the assay and were probably due to individual variations in the plasma level of cholesterol esterase.

The possible influence of cholesterol esterase in lipoprotein levels in human subjects was investigated by comparing plasma cholesterol and lipoprotein levels with the level of cholesterol esterase activity present in each sample. Thirty healthy human volunteers, ages 19–45, were recruited for the initial determination. The nature and consequence of the study were explained to the volunteers and informed consent was obtained. The subjects were of both genders with 57% male and 43% female. All donors were fasted overnight for at least 14 h until blood was drawn at 9 a.m. An aliquot of each plasma sample was used for cholesterol and lipoprotein determinations while 0.6 ml of each plasma sample was centrifuged at $d = 1.21$ g/ml to prepare lipoprotein deficient plasma. The cholesterol esterase was found exclusively in the lipoprotein deficient plasma and no enzyme was associated with lipoproteins after ultracentrifugation. Therefore, the lipoprotein deficient plasma at the $d > 1.21$ g/ml fraction was used for all cholesterol esterase activity measurements. The results, as shown in Fig. 1, revealed a significant correlation between bile salt-stimulated cholesterol esterase activity and cholesterol level in plasma ($r = 0.694$, $p = .0001$).

The elevated cholesterol esterase level may be a result of increased plasma cholesterol concentration. Alternatively, elevated level of cholesterol esterase may be a causal factor and contributes to plasma cholesterol homeostasis in human subjects. These possibilities were investigated by examining the plasma cholesterol esterase activity in two homozygous familial hypercholesterolemic subjects. The results showed that, despite the severe elevation in their plasma cholesterol levels, due to defects in LDL receptor function, the cholesterol esterase levels in these subjects were similar to those observed with healthy individuals (Fig. 1). This observation contradicts the first hypothesis and suggests the exciting possibility that high level of plasma cholesterol esterase activity, at least in normolipidemic and moderately hyperlipidemic subjects, may contribute to a higher steady state cholesterol level in human plasma.

The relationship between plasma cholesterol and cholesterol esterase activity was further explored by determining the relationship between cholesterol esterase activity with

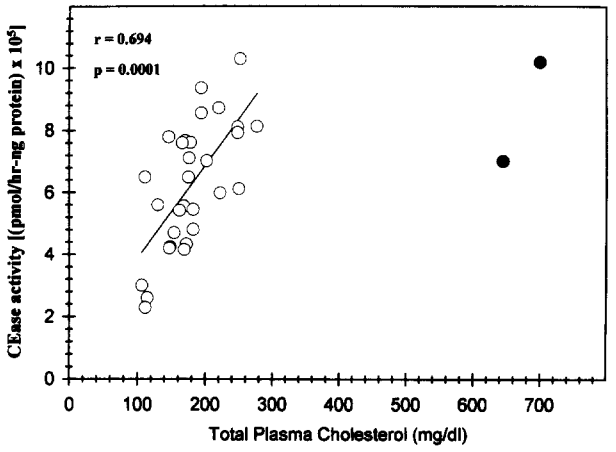

Fig. 1. Correlation of human plasma cholesterol levels with cholesterol esterase activity in 30 healthy donors (open circles) and two homozygous Type II familial hypercholesterolemic patients (filled circles). Total plasma cholesterol was determined by a hydrolase/oxidase colorimetric method in a Hitachi 717. Lipoprotein deficient plasma, prepared by ultracentrifugation at $d > 1.21$ g/ml, were used to determine cholesterol esterase activity. The reaction mixture containing 50 mM Tris-HCl (pH 7.5), 33 mM cholate, and 1.96 nmol of cholesteryl [^{14}C]oleate. The samples were incubated for 1 hr at 37°C. The reaction was terminated by adding 3.25 ml of methanol/chloroform/heptane (1.41:1.25:1.0; v/v/v) and 1.05 ml of 50 mM sodium carbonate and 50 mM sodium borate (pH 10). After centrifugation of the samples for 35 min at 1500$\times g$, 500 μl of the aqueous fraction was removed for liquid scintillation counting. Cholesterol esterase activity was determined based on the appearance of [^{14}C]oleate. The correlation of plasma cholesterol with bile salt-activated cholesterol esterase activity in the plasma of normolipidemic individuals was analyzed by linear regression.

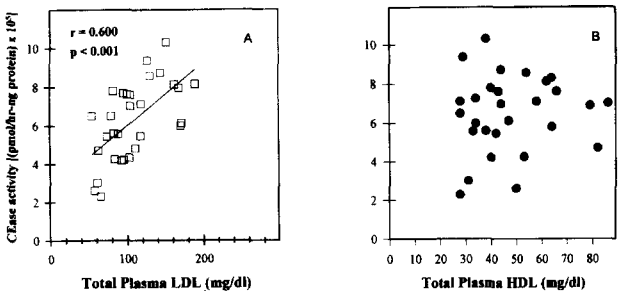

Fig. 2. Correlation of plasma cholesterol esterase activity with LDL-cholesterol (A) and HDL-cholesterol (B) levels in normal human subjects. Lipoprotein deficient plasma from 30 healthy donors (open triangles) were used to determine cholesterol esterase activity, based on the hydrolysis of cholesteryl [^{14}C]oleate. The level of HDL-cholesterol was determined after phosphotungstate/$MgCl_2$ precipitation and centrifugation to remove the LDL and VLDL. The level of LDL-cholesterol was calculated using the Friedenwald equation: [LDL-Cholesterol] = [Total Cholesterol] − [HDL-Cholesterol] − [VLDL-Cholesterol], where [VLDL-Cholesterol] is estimated by [triglyceride]/5.

LDL and HDL levels. In these experiments, subjects with known genetic defects in the lipoprotein metabolic pathway, such as the Type II familial hypercholesterolemic patients, were excluded and only healthy normolipidemic volunteers were tested. A positive correlation was observed between cholesterol esterase activity with plasma LDL level ($r = 0.600$, $p < 0.001$) (Fig. 2A). However, the relationship between plasma cholesterol esterase and HDL level was not statistically significant (Fig. 2B).

Additional experiments were performed to ascertain the possibility that plasma cholesterol esterase level may be a contributing factor in determining LDL particle size. Two separate preparations of LDL were incubated with or without purified cholesterol esterase in vitro using the procedure described by Aviram et al. [23]. Because bile salt concentration in plasma is below that required for cholesterol esterase stimulation, these experiments were performed in the absence of bile salt to mimic the physiologic environment. The samples were then applied to nondenaturing gradient gel electrophoresis for analysis. The results indicated that, after 1 h of incubation, the purified cholesterol esterase converted most of the larger LDL subfractions to the smaller LDL species (Fig. 3). Prolonged incubation of LDL with cholesterol esterase resulted in the complete conversion of large LDL to small LDL particles. These small LDL appeared to be the end product of cholesterol esterase-hydrolyzed LDL, since lipoproteins smaller than 23 nm were not observed.

Table 2
Lipid composition of control and cholesterol esterase-hydrolyzed LDL

Lipid	Control LDL (mg/mg protein)	CEase-LDL (mg/mg protein)
Free cholesterol	0.45 ± 0.11	0.70 ± 0.15
Cholesteryl esters	1.50 ± 0.33	1.20 ± 0.40
Phospholipids	1.00 ± 0.15	0.95 ± 0.20
Triglycerides	0.20 ± 0.08	0.17 ± 0.10

The mechanism of cholesterol esterase induced changes in LDL size was investigated by determining lipid composition of the LDL before and after incubation with cholesterol esterase or with a mutagenized inactive enzyme [24,25]. A consistent decrease in LDL cholesteryl ester, with concomitant increase in free cholesterol, was observed after treatment with native cholesterol esterase. In three separate determinations using different LDL preparations, the ratio of cholesteryl ester to free cholesterol in the LDL changed from an average of 3.33 to 1.70 after

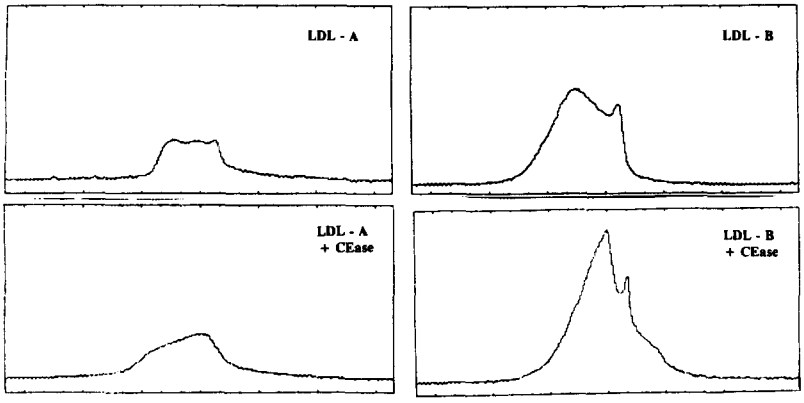

Fig. 3. Effects of cholesterol esterase on LDL subfractions. The LDL fraction ($d = 1.019–1.063$ g/ml) was isolated from plasma of two subjects. Cholesterol esterase was purified from rat pancreas as described (25). The LDL (30 μg protein) were incubated with (bottom panels) or without (top panels) 3 μg of cholesterol esterase for 1 h at 37°C. Six μg of LDL protein from Subject 1 and 30 μg of LDL protein from subject 2 were electrophoresed overnight in a 2–16% nondenaturing gradient polyacrylamide gel. The gel was stained with Coomassie brilliant blue R-250 and destained with methanol/acetic acid. The LDL phenotype was determined by scanning from the top (left margin) to the bottom (right margin) of the gel. The LDL particle sizes were determined by comparison with a standard curve composed of apoferritin (12.2 nm), thyroglobulin (17 nm), and carboxylated latex beads (38.1 nm).

treatment with the native enzyme (Table 2). However, phospholipid and triglyceride contents in the LDL remained essentially unchanged after incubation with the active cholesterol esterase. Incubation of LDL with the inactive $His^{435} \rightarrow Gln^{435}$ mutant form of cholesterol esterase [24,25] did not change the lipid composition of the LDL nor altered its electrophoretic mobility on nondenaturing gradient gels (data not shown). Thus, the cholesterol esterase-induced alteration in LDL particle size requires hydrolytic activity of the enzyme and is unrelated to the lipid transfer properties of cholesterol esterase [26]. The effect of cholesterol esterase on LDL particle size is most likely due to its ability to alter the composition of surface and core components of the lipoprotein. Alternatively, free fatty acids generated from cholesterol esterase hydrolysis of the cholesteryl esters may also alter the size of the lipoprotein.

Regardless of the precise mechanism for cholesterol esterase induced change in LDL particle size, the ability of cholesterol esterase to convert large LDL particles to their smaller counterparts is provocative. Recent studies have demonstrated that LDL particles are biochemically heterogeneous in size and composition [27]. The heterogeneity in LDL can be separated into two distinct phenotypes, denoted A and B, based on gradient gel electrophoresis. Subjects with phenotype A is characterized by a preponderance of large LDL particles while subjects with the B phenotype is characterized by a preponderance of smaller LDL [reviewed in [28]]. The LDL phenotype B has been linked to an increased risk of coronary heart disease [28]. Therefore, the ability of cholesterol esterase to convert large LDL to smaller LDL subspecies, and the relationship between plasma cholesterol esterase and LDL levels, suggest that plasma cholesterol esterase level may be a potential risk factor for atherosclerosis. Although the precise mechanism by which elevated cholesterol esterase leads to LDL accumulation in plasma remains unknown, it is possible that the cholesterol esterase may facilitate the conversion of VLDL and IDL particles to LDL. The ability of cholesterol esterase to hydrolyze large lipoproteins to their smaller counterparts is consistent with this hypothesis. Alternatively, cholesterol esterase may also inhibit LDL clearance from circulation. The latter hypothesis is supported by a report demonstrating reduced cellular uptake of LDL after its hydrolysis by bacterial cholesterol esterase [23]. Regardless of the mechanism of cholesterol esterase induced LDL accumulation, this study points to the possibility of using plasma cholesterol esterase level measurement as a diagnostic marker for early identification of subjects at risk for premature atherosclerosis. Inhibitors for cholesterol esterase may also be employed as a therapeutic intervention strategy to reduce the risk of coronary heart disease. Additional detailed studies exploring the relationship between cholesterol esterase genotype with plasma cholesterol and LDL level, and their mode of inheritance of these traits, are also warranted.

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